Recognition of a Nucleic Acid Base by Tryptophan-Containing Peptides: Spectroscopic Comparison of the Interaction of Trp–Gly–Gly–Glu and Trp–Gly–Gly–Gln with 7-Methylguanine Base[1)]

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As a part of a study to elucidate the functional difference between Glu and Gln side-chains in terms of the recognition of guanine base by tryptophan-containing peptides *via* cooperative stacking and hydrogen-bond pairing interactions, the binding of 7-methylguanine to Trp–Gly–Gly–Glu and Trp–Gly–Gly–Gln was examined by fluorescence and ^{1}H-NMR methods. Comparison of fluorescence experiments showed a binding preference for Trp–Gly–Gly–Glu over Trp–Gly–Gly–Gln. The analyses of the downfield and upfield shifts of the C2 amino and N7 methyl protons of 7-methylguanine base showed there was hydrogen-bond pairing of Glu and Gln side chains with the base and a stacking interaction of the Trp residue with the base, respectively. However, the hydrogen-bond pairing was more effective in the case of the Glu residue than the Gln residue, indicating the preference of the carboxyl group over the carbamoyl group to form hydrogen-bond pairing with the guanine base.

Keywords Trp–Gly–Gly–Glu; Trp–Gly–Gly–Gln; 7-methylguanine base; hydrogen-bond pairing; stacking interaction; fluorescence; ^{1}H-NMR

Selective recognition of a specific nucleotide or nucleic acid sequence by an enzyme is one of the most basic biological functions. As part of peptide design studies seeking high selectivity for a target nucleic acid base, the binding abilities of a series of tryptophan-containing peptides have been investigated by spectroscopic methods.[1,2)] As a result, it was established that Trp–Gly–Gly–Glu exhibits the highest selectivity for 7-methylguanine (m^7G) base among the Trp–$(Gly)_n$–Glu peptides ($n=0$—3),[2c)] where the intimate coupling of aromatic stacking and hydrogen-bond pairing interactions plays a very important role in such a specificity.

Concerning the hydrogen-bond pairing of polar amino acid side-chains to m^7G base, three different types are possible under physiological conditions (Fig. 1). Therefore, it would be expected that a peptide such as Trp–Gly–Gly–Gln would also show the same high selectivity for m^7G base as Trp–Gly–Gly–Glu. However, hydrogen-bond pairing *via* the acidic amino acid is generally observed in guanine base recognition by proteins such as RNase T_1,[3)] elongation factor-Tu[4)] and c-H-ras oncogene p21.[5)] This may mean that the carboxyl group is superior to the carbamoyl group as far as hydrogen-bond pairing with guanine base is concerned. In order to investigate this possibility under conditions involving the fixation of m^7G base by the stacking interaction with the Trp indole ring, a spectroscopic comparison of the interaction of Trp–Gly–Gly–Glu and Trp–Gly–Gly–Gln with m^7G base was carried out.

Experimental

Materials The peptides were synthesized by the usual liquid phase peptide condensation technique and purified by gel chromatography on Sephadex LH20 and by HPLC on an octadecyl silica (ODS) column (Capcell Pack C18, Shiseido) as described in a previous paper.[1)] 7-Methylguanosine 5′-phosphate (m^7GMP) and 7-methylguanosine (m^7Guo) were synthesized as formate salts from GMP and Guo, respectively, according to Kamiichi *et al.*[6)]

Spectroscopic Measurements Fluorescence spectra were measured on a JASCO FP-770F spectrometer (Nihon Bunko) equipped with a Hg–Xe arc lamp; a 10-nm slit and 1-cm path length were used. The temperature of the sample solution was kept at 20 °C by circulating water at a constant temperature. The intensities of the emission spectra excited at 290 nm were measured at a λ_{max} near 353 nm. The sample preparation using 20 mM Tris–HCl (pH = 7.5) buffer, the fluorescence experiments and the determination of association constants (K_a) between peptide and m^7GMP pairs by means of the Eadie–Hofstee equation[7)] were carried out according to a previous publication.[1)]

^{1}H-NMR measurements were performed on a Varian XL-300 spectrometer (300 MHz for proton) at 20 °C. In order to monitor the chemical shift changes of C2 amino and N7 methyl protons of m^7G base, dimethylsulfoxide (DMSO)-d_6 was used as a solvent and the chemical shifts were measured as differences from an internal standard, tetramethylsilane (TMS). Because of better solubility, m^7Guo was used

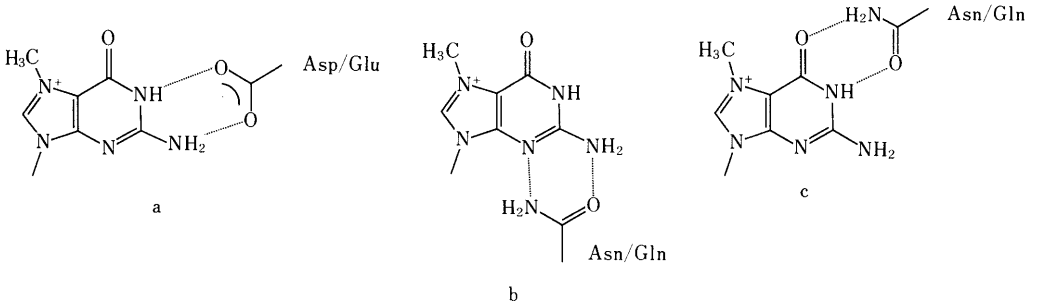

Fig. 1. Possible Hydrogen-Bond Pairings between m^7G Base and Polar Amino Acid Side-Chains

Hydrogen bonds are represented by dotted lines.

instead of m^{7}GMP. The determination of the association constants from the chemical shift changes of m^{7}Guo C2 amino and N7 methyl protons[8,9] by means of the Eadie–Hofstee equation were carried out according to a previous publication.[1]

The 1 : 1 stoichiometry of the m^{7}Guo-peptide interaction was confirmed by the Job plot using the chemical shift change of m^{7}Guo C2 amino and N7 methyl protons.[10]

Each experiment was carried out three times and mean values calculated.

Results and Discussion

It is well known that the fluorescence intensity of the Trp indole ring decreases following a stacking interaction with nucleic acid base.[11] Thus, the fluorescence quenching of the Trp residue in a peptide can be measured as a function of m^{7}GMP concentration. Figure 2 shows the Eadie–Hofstee plots of m^{7}GMP titration against 5 μM peptide; the association constants obtained by least-squares fitting are given in Table I.

The results show that the degree of binding between Trp–Gly–Gly–Glu and m^{7}GMP is about three times that of Trp–Gly–Gly–Gln. Since this difference is mainly due to the interaction of the Glu/Gln residue with m^{7}GMP,[12] it can be said that (a) the stacking interaction between the N-terminal Trp and m^{7}GMP is highly cooperative with the interaction mode of the C-terminal residue with the nucleotide and (b) the interaction of the nucleotide with the Glu residue favours a stacking interaction more than with Gln.

In order to consider the possible binding modes of the two peptides with m^{7}G base, the changes in m^{7}G C2 amino and N7 methyl proton chemical shifts were examined as a function of peptide concentration. The results are shown in Fig. 3, and the association constants evaluated from the slopes of the respective Eadie–Hofstee plots are listed in Table I.

The C2 amino protons of m^{7}Guo exhibited a downfield shift proportional to the peptide concentration (Fig. 3a). This is generally accepted as an indication of the participation of this amino group in hydrogen-bond formation with the peptide acceptor group. The degree of downfield shift is more pronounced for Trp–Gly–Gly–Glu than for Trp–Gly–Gly–Gln, and this could be due to the preferential hydrogen-bonding of m^{7}GuO with the C-terminal Glu rather than the Gln residue. Since the carbamoyl NH_2 of the Gln side-chain also shifted downfield upon interaction with m^{7}Guo, the base pairing shown in Fig. 1b could be possible in the case of Trp–Gly–

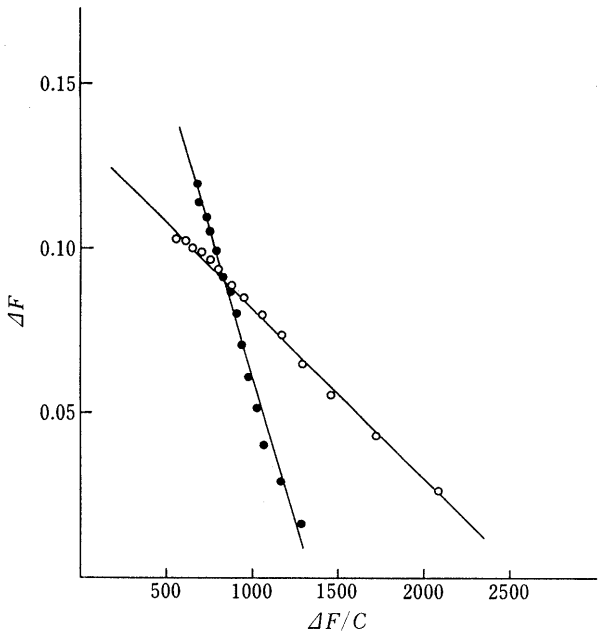

Fig. 2. Eadie–Hofstee Plots of Trp Fluorescence Quenchings in Trp–Gly–Gly–Glu (—○—) and Trp–Gly–Gly–Gln (—●—) as a Function of m^{7}GMP Concentration in Tris–HCl buffer (pH 7.5)

Table I. Association Constants (K_a, M^{-1}) between m^{7}G Derivatives and Peptides Determined by Fluorescence and ^{1}H-NMR[a]

	Fluorescence	^{1}H-NMR	
		C2-NH_2	N7-CH_3
	m^{7}GMP	m^{7}Guo	
Trp–Gly–Gly–Glu	$1.80(7)\times10^4$	$2.4(2)\times10^2$	$6.9(5)\times10$
Trp–Gly–Gly–Gln	$5.26(8)\times10^3$	$1.3(2)\times10^2$	$7.4(6)\times10$

a) The standard errors are given in parentheses.

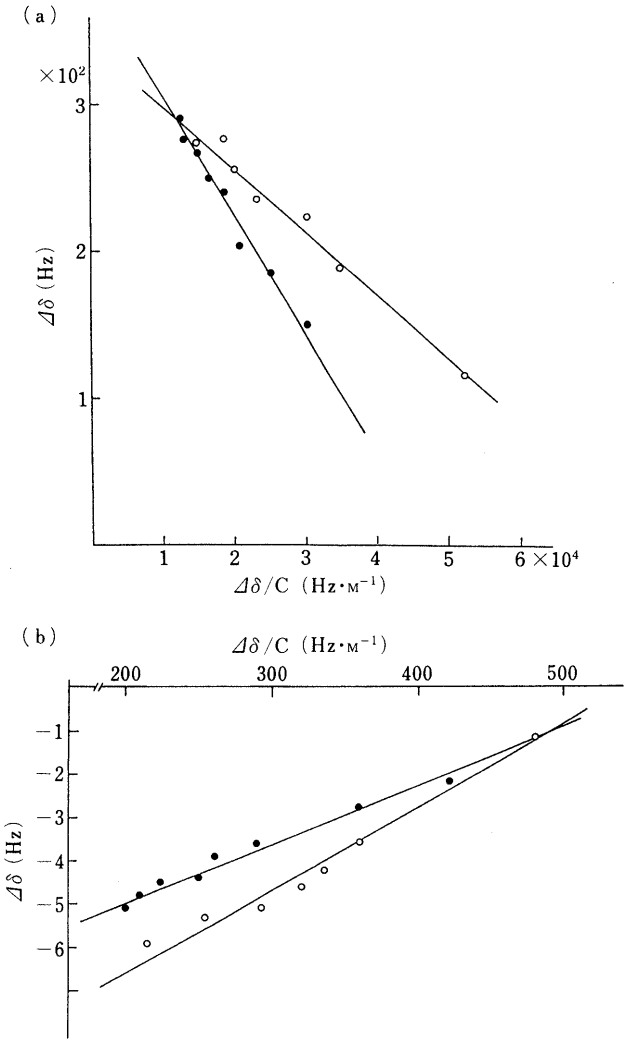

Fig. 3. Eadie–Hofstee Plots of Chemical Shift Changes of m^{7}Guo C2 Amino (a) and N7 Methyl (b) Protons as a Function of Peptide Concentration in DMSO-d_6

○, Trp–Gly–Gly–Glu; ●, Trp–Gly–Gly–Gln.

Gly–Gln. Concerning the hydrogen-bonding between the Glu carboxyl group ($pK_a = 3.86$) and m^7G base, the hydrogen-bond pairing as shown in Fig. 1a would certainly be most possible, as demonstrated by X-ray crystallographic[13] and NMR[14] analyses of related complexes, although no direct evidence was found in this NMR experiment.[15]

On the other hand, the N7 methyl protons exhibit an upfield shift, depending on the peptide concentration (Fig. 3b). This is mainly due to the ring current effect produced by the m^7G base–Trp indole ring stacking interaction. The association constants obtained for both peptides indicate nearly the same stacking behavior under the solution conditions used, and are not large enough to establish any interaction preference for either peptides towards the guanine base; the discrepancy between this and the fluoresence results could be due to the use of different concentrations, different solvents used for the measurements,[16] and/or the overestimation by the fluoresence spectroscopy.[17]

The present study clearly indicates the superior hydrogen-bond pairing ability of the Glu carboxyl side-chain with respect to m^7G base compared with the Gln carbamoyl chain, as well as the importance of its close involvement with the Trp stacking interaction for base recognition.

References and Notes

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8) The H8 proton of m^7Guo was not suitable as an indicator for the stacking interaction with the Trp residue because of its cationic character caused by N7 methylation.[6] The N1 imino proton was not observed because of the fast H–D exchange and/or enolate tautomerism[9] due to N7 methylation.
9) S. E. Carberry, R. E. Rhoads, D. J. Goss, *Biochemistry*, **28**, 8078 (1989).
10) According to the Job plot (P. Job, *Comput Rend.*, **180**, 928 (1925)), the 1 : 1 stoichiometry of the peptide–m^7Guo was determined by plotting the m^7Guo C2 amino and N7 methyl proton chemical shifts as a function of molar fraction $\{[m^7Guo]/[peptide + m^7Guo]\}$, where the total concentration of $[m^7Guo] + [peptide]$ was kept constant (= 20 mM); the Job plot exhibited a maximum at a molar fraction of $[peptide]/[m^7Guo + peptide] = 1/2$.
11) C. Hélène, J. Maourizot, *CRC Crit. Rev. Biochem.*, **10**, 213, (1981).
12) Since the superiority of Trp–Gly–Gly–Glu over Trp–Gly–Gly–Gln was also observed in terms of the interaction of m^7Guo, the effect of the interaction of these peptides with the m^7GMP phosphate group would be negligible.
13) *a*) T. Ishida, M. Katsuta, M. Inoue, Y. Yamagata, K. Tomita, *Biochem. Biophys. Res. Commun.*, **115**, 849 (1983); *b*) T. Ishida, M. Doi, H. Ueda, M. Inoue, G. M. Sheldrick, *J. Am. Chem. Soc.*, **110**, 2286 (1988); *c*) T. Ishida, M. Doi, M. Inoue, *Nucleic Acid Res.*, **16**, 6175 (1988).
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15) If m^7Guo takes an enolate form due to N7 methylation, as has been supposed by Carberry, *et al.*,[9] only a hydrogen bond *via* the base C2 amino group is possible in Fig. 1a. However, all the crystals containing the m^7G base exhibited the N1 imino form of the base.[13]
16) It should be noted that the stacking interaction tends to function effectively in aqueous solution, while the hydrogen-bond interaction is apt to become a major stabilizing factor in organic solvents such as DMSO.
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